농학석사학위논문

Antimicrobial metabolites from *Streptomyces* sp. MBL39 isolated from marine-derived sediment

해양방선균 *Streptomyces* sp. MBL39 유래의 항균활성물질 연구

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Abstract

Antimicrobial metabolites from *Streptomyces* sp. MBL39 isolated from marine-derived sediment

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Bioactive secondary metabolites from marine organisms are now generally considered to be promising and developable drug sources. As part of ongoing efforts to identify new bioactive compounds from marine actinomycetes, 373 of microorganisms were isolated from 77 of marine samples collected at the shore of Micronesia. Among them, the bacterial strain MBL39 which was isolated from mangrove plants exhibited the most significant

antimicrobial activity. This strain was identified as *Streptomyces* sp. MBL39 by 16S rRNA sequence analysis. The crude MeOH extract of the bacterial culture broth showed strong antibacterial activities against several Gram-positive and Gram-negative bacteria. Therefore, this extract was sequentially fractionated with *n*-hexane, ethyl acetate, *n*-butanol, and water. Bioassay-guided separation of the ethyl acetate fraction using various chromatographic techniques yielded seven compounds. Among them, compound 1, 2, 4, and 5 showed potent antibacterial activity against Gram-positive and Gram-negative bacteria with MIC values in the range of 3.1-25 μg/ml. The structure of compound 4 was defined as oleamide on the basis of mass spectrometry and detailed 2D NMR spectroscopic data. The in vitro inhibitory activity of the isolated compounds was also accessed against several target enzymes such as isocitrate lyase, a key enzyme of the glyoxylate cycle, and sortase A, a bacterial surface protein anchoring transpeptidase. For the more detailed structure of compound 1, 2, and 5, NMR analysis should be performed.

Keywords : Marine actinomycete, *Streptomyces*, Oleamide, Antibacterial activity

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1. Introduction

The actinomycetes are the most economically and biotechnologically valuable Gram-positive bacteria. They are responsible for producing about half of the discovered bioactive secondary metabolites such as antibiotics, antitumor agents, immunosuppressive agents and enzymes(1). Because of the excellent track record of actinomycetes in this regard, a significant amount of effort has been focused on the successful isolation of novel actinomycetes from terrestrial sources for drug screening programs in the past fifty years. Unfortunately, beginning in the late 1980s, the rate of discovery of new drug candidates from terrestrial actinomycetes began to decrease(2). With the dramatic increase in the emergence of drug-resistant infectious diseases, it is crucial that new groups of actinomycetes from unexplored or underexploited habitats be pursued as sources of novel bioactive secondary metabolites.

Although the diversity of life in the terrestrial environment is extraordinary, the greatest biodiversity is in the oceans. About 70% of earth' s surface is covered with water and it comprises 5,00,000 live species divided into 30 different phyla(3). The world ocean has a large source which still has not been utilized. It holds an outstanding potential for discovery and development

of bioactive natural products. As marine environmental conditions are extremely different from terrestrial ones, it is surmised that marine-derived actinomycetes have different characteristics from those of terrestrial counterparts and, therefore, might produce different types of bioactive compounds. The research to date supports this hypothesis and it has been shown that marine actinomycetes produce novel types of new secondary metabolites. Many of these metabolites possess novel biological activities and have the potential to be developed as therapeutic agents.

Actually, numerous novel metabolites such as salinosporamide, marinomycins, arenimycin, caboxamycin, *etc* have been derived from marine actinomycetes in the past few years(4). Salinosporamide A is a novel rare bicyclic beta-lactone gamma-lactam isolated from an obligate marine actinomycete, *Salinispora tropica*. Salinosporamide A is an orally active proteasome inhibitor that induces apoptosis in multiple myeloma cells with mechanisms distinct from the commercial proteasome inhibitor anticancer drug Bortezomib(38).

Salinipyrones A and B are new polyketides isolated from a phylogenetically unique strain of the obligate marine actinomycete, *Salinispora pacifica*. The biological activity of salinipyrones A and B did not show appreciable antibacterial activity against drug resistant human pathogens. However, salinipyrone A displayed moderate inhibition of interleukin-5

production by 50% at 10 μg/mL without measurable human cell cytotoxicity(39).

Lodopyridone is a unique alkaloid produced by a marine *Saccharomonospora* sp. isolated from marine sediments collected at the mouth of the La Jolla Submarine Canyon. Lodopyridone possess activity against the human colon adenocarcinoma ell line HCT-116 with an IC_{50} of 3.6 μM(40).

Arenimycin is a new antibiotic belonging to the benzo [α]naphthacene quinone class produced by the obligate marine actinomycete, *Salinispora arenicola*. This new structural derivative is the first report of this class of antibiotics from this strain *S. arenicola*. Arenimycin has effective antibacterial activity against rifampin- and methicillin-resistant *Staphylococcus aureus* and it exhibits potent antimicrobial activities against drug -resistant *Staphylococci* and other Gram-positive human pathogens(41).

Additionally, sequencing marine actinomycete genomes may provide insights useful in the discovery of novel agents(5). Now examining the natural product profiles of the underexploited marine-derived actinomycetes has become a research hot spot in drug discovery.

Glyoxylate cycle is well known for anaplerotic variant of the tricarboxylic acid (TCA) cycle, which is the conversion of two molecules of acetyl-CoA to succinate(Fig. 1). It is well known that the glyoxylate cycle operates in bacteria, fungi, some

protein and plants. In glyoxylate cycle, the net assimilation of carbon from C_2 compounds allow microorganisms to supplement the pool of TCA cycle intermediates necessary for many biosynthetic processes including gluconeogenesis. Most of all, pathogenic microorganisms utilize the glyoxylate cycle during infection(33).

Isocitrate lyase(ICL) and malate synthase(MLS) are key enzymes in glyoxylate cycle. ICL cleaves isocitrate to glyoxylate and succinate and MLS condenses glyoxylate with acetyl-CoA to malate. Several researches support that ICL plays a role as an essential enzyme in pathogenesis. In case for *Candida albicans*, an important pathogenic fungus of humans, ICL is strongly upregulated. When *C. albicans* is exposed to macrophages and human neutrophils, the fact that ICL is induced is confirmed by previous experiment data(34).

ICL is considered to be an attractive drug target for the development of effective antimicrobial drugs against a wide range of pathogens, including both bacteria and fungi. To date, several ICL inhibitors including 3-nitropropionate, 3-bromopyruvate, mycenon, oxalate and itaconate have been identified(35,36). However, these inhibitors are not appropriate for testing in vivo since they have toxicities and weak ICL inhibitory activities. Therefore, development of novel ICL inhibitors with suitable pharmacological properties are required.

As part of my ongoing research on new antimicrobial

compounds from marine-derived microorganisms, 373 of microbes were isolated from marine smaples. Among them, strain MBL39, which was isolated from mangrove plants, was found to exhibit significant antibacterial activity. MBL39 was grown on a variety of media under various conditions, some of which yielded potent antibacterial activity. Guided by this activity, I isolated seven compounds by a diversity of chromatographic methods. Four of the seven compounds possess antibacterial activities, with MIC values of 6.25 ~ 25 μg/ml, against *Staphylococcus aureus* ATCC 6538p, *Bacillus subtilis* ATCC 6633, *Kocuria rhizophila* NBRC 12708, *Salmonella enterica* ATCC 14028 and *Proteus hauseri* NBRC 3851. However, they did not show inhibitory activity against ICL and sortase A(SrtA). These compounds were subsequently purified, and the structure of one compound was determined by combined spectroscopic methods including 2D-NMR. As a result, compound 4 was identified to be oleamide. Oleamide has great potential as a slip agent and a chemopreventive agent against Alzheimer's disease.

2. Materials and Methods

2.1. Isolation of MBL39

Strain MBL39 was isolated from mangrove plants which were collected at the shore of Micronesia in December 2011. To isolate MBL39 from marine samples, two different media were used(6). HV agar medium consisted of 1 g of Humic acid dissolved in 10 ml of 0.2 N NaOH, 0.5 g of Na_2HPO_4, 1.7 g of KCl, 0.05 g of $MgSO_4 \cdot 7H_2O$, 0.01 g of $FeSO_4 \cdot 7H_2O$, 0.02 g of $CaCO_3$, B-vitamins(0.5 mg each of thiamine-HCl, riboflavin, niacin, pyridoxin-HCl, inositol, Ca-pantothenate, p-aminobenzoic acid, and 0.25 mg of biotin), 18 g of agar, and 1 L of distilled water. Actinomycete isolation agar medium consisted of 22 g of Himedia Actinomycete Isolation Agar, 5 ml of glycerol, and 1 L of distilled water. 50 mg of cycloheximide was added to both of the media after sterilization.

2.2. Identification of MBL39

Genomic DNA of MBL39 was extracted using a i-genomic BYF DNA mini kit (intron biotech, Korea). A region of approximately 1500 bp from the 16S rDNA was amplified using the primers F27 (5'-AGAGTTTGATCMGGCTCAG-3') and R1525 (5'-AAGGAGGTGWTCCARCC-3')(7). Amplified 16S rDNA was cleaned using PCR product purification kit (intron biotech, Korea) and cloned into pGEM-T Easy vector following manufacturer's instructions. Sequence of 16S rDNA was analysed by an Applied Biosystem model 373A automatic sequencer and a BigDye Terminator Cycle Sequencing kit (Perkin-Elmer applied Biosystems). 16S rDNA sequences matched with sequences in the nucleotide database using the BLAST algorithm with default parameters within NCBI (National Center for Biotechnology Information) to identify related 16S rDNA sequences. For the construction of phylogenetic trees, sequence was aligned with Clustal X v. 1.83 and analyzed with BioEdit v. 7.2.3(8-9). Trees were derived from the distance matrices using neighbour-joining method by MEGA5.2 program(10).

2.3. Cultivation and Extraction of MBL39

To confirm antimicrobial activity, six different broth media were used. GTYB medium consisted of 10 g of glucose, 2 g of tryptone, 1 g of yeast extract, 1 g of beef extract, and 1 L of distilled water(11). Bennett medium consisted of 10 g of glucose, 2 g of NZ-amine, 1 g of yeast extract, 1 g of beef extract, and 1 L of distilled water(12). SYP medium consisted of 10 g of starch, 4 g of yeast extract, 2 g of peptone, and 1 L of distilled water(13). GPY medium consisted of 10 g of glucose, 3 g of yeast extract, 5 g of peptone, and 1 L of distilled ater(14). M2 medium consisted of 4 g of glucose, 4 g of yeast extract, 10 g of malt extract, and 1 L of distilled water(15). YPM medium consisted of 4 g of mannitol, 2 g of yeast extract, 2 g of peptone, and 1 L of distilled water(16). All media were sterilized by autoclaving at 120℃ for 20 min. MBL39 was cultured in 100 ml of six broth media at 28℃ with shaking at 120 rpm for 5 days. The fermented culture was filtered by filter paper (300mm, qualitative, Advantec, Japan) to separate the mycelia. The filtrate was extracted with ethyl acetate three times. The EtOAc extract was evaporated *in vacuo* at 40℃ and then the residue was dissolved in dimethyl sulfoxide and stored at −20℃.

2.4. Isolation of compounds from MBL39

MBL39 was cultured in 96 × 0.5 L volumes of YPM media (2 g mannitol, 1 g yeast extract, 1 g peptone, 0.5 L of distilled water) while shaking at 120 rpm for 7 days at 28℃. The fermented culture (48 L) was filtered by filter paper and concentrated under reduced pressure. The residue was repeatedly extracted with MeOH. The MeOH extracts (76.78 g) were successively partitioned between H_2O and hexane, ethyl acetate, and butanol and then layer was dried. The EtOAc extract (1.12 g) was subjected to C_{18} reversed-phase vacuum flash chromatography using mixtures of methanol and water as eluents (elution order : H_2O, 10%, 20% aqueous MeOH, and 100% MeOH) and finally 100% acetone. The 100% MeOH fraction (106.2 mg) was separated by C_{18} reversed-phase HPLC (Agilent Eclipse Plus column, 250×4.6mm, 90% aqueous Methanol), then purified by C_{18} reversed-phase HPLC (Agilent Eclipse Plus column, 250×4.6mm, 85% aqueous Methanol) to yield 0.3, 0.5, 0.7, 1.0, 0.7, 0.9, 0.4 mg of 1-7, respectively (Fig. 3).

2.5. Antibacterial activity assay

The following 6 microorganisms, obtained from the stock culture collection at American Type Culture Collection (ATCC) (Rockville, MD, U.S.A.) and NITE Biological Resource Center (NBRC) (Tokyo, Japan), were used in this study : *Staphylococcus aureus* ATCC 6538p, *Bacillus subtilis* ATCC 6633, *Kocuria rhizophila* NBRC 12708, *Salmonella enterica* ATCC 14028, *Proteus hauseri* NBRC 3851, *Escherichia coli* ATCC 35270. The antibacterial activity was determined by the two-fold microtiter broth dilution method. Dilution of test compound dissolved in dimethyl sulfoxide (DMSO) were added to each well of 96-well microtiter plate containing fixed volume of m plate count broth (Difco). The concentration of compounds ranges 100 to 0.39 μg/ml. Each well was inoculated with an overnight culture of bacteria (10^5 CFU/ml), and incubated at 37℃ for 16 hours. The minimum inhibitory concentration (MIC) was determined as the lowest concentration of test compound that inhibited bacterial growth(17). Ampicillin was used as a reference compound.

2.6. Antifungal activity assay

Aspergillus fumigates HIC 6094, *Trichophyton rubum* IFO 9185, *Trichophyton mentagrophytes* IFO 40996 and *Candida albicans* ATCC 10231 as microorganisms were used in antifungal activity assay. The antifungal activities of compounds were determined by following broth dilution method M27-A2, which was proposed by the National Committee for Clinical Laboratory Standards(18). Each compound was diluted with potato dextrose broth (Difco) to prepare serial two-fold dilutions in the range of 100 to 0.39 μg/ml. One hundred microliters of the broth containing about 10^4 spore/ml except *C. albicans* was added to each well of a 96-well microtiter plate. In case of *C. albicans*, about 10^2 spore/ml was contained in one hundred microliters of the broth. the MIC was determined after 48 hours incubation at 28℃. Amphotericin B was used as a reference compound(19).

2.7. Expression and purification of ICL protein

In a previous work, the cloning and purification of ICL from the genomic DNA Candida albicans (ATCC 10231) were carried out as described previously(37). The positive transformants were grown in LB medium containing ampicillin (50 μg/ml) at 37 ℃ for 16 hours (O.D.600 = 1~2). The pre-incubated cells (2 ml) were inoculated into 400ml of LB broth containing ampicillin and incubated with shaking at 37 ℃ until O.D.600 reached 0.5 approximately. At this point L-arabinose was added to the medium to a final concentration of over 0.02% and an additional incubation was performed at 25 ℃ for 8 hours to induce the expression of ICL proteins. Growth at the low resulted in improved solubility of the recombinant system are fused to His-Patch(HP)-thioredoxin at the N-terminal for the simplified purification. Since HP-Thioredoxin has a Ni-binding property at pH 7.2, the expressed ICL could easily be purified by Ni-NTA affinity column chromatography.

2.8. ICL activity assay

A 1-ml aliquot of the reaction mixture contained 20 mM sodium phosphate buffer (pH 7.0), 1.27 mM threo-DL (+) isocitrate, 3.75 mM $MgCl_2$, 4.1 mM phenylhydrazine, and 2.5 μg/ml purified ICL. The reaction was performed at 37 ℃ for 30 min with and without prescribed concentration of inhibitor dissolved in DMSO (final concentration, 1%). The formation of glyoxylate phenylhydrazone was followed spectrophotometrically at 324 nm. The effect of inhibitor on ICL was calculated as a percentage, relative to solvent-treated control, and the IC_{50} values were calculated using nonlinear regression analysis (percent inhibition versus concentration). 3-Nitropropionate is used as a positive control.

2.9. NMR & MS analysis

NMR spectra were recorded in CD_3OD solutions on Bruker AVANCE600 spectrometer. Both proton and carbon NMR spectra were measured at 600 MHz. Mass spectra were provided by the National Center for Inter-University Research Facilities and the National Instrumentation Center for Environmental Management, Seoul, Korea.

3. Results

3.1. Isolation of MBL39

373 of microbes were isolated from 77 marine samples. Among them, strain MBL39, which was isolated from mangrove plants, was found to exhibit significant antibacterial activity. MBL39 was grown on a variety of media at 28℃ with shaking at 120 rpm for 5 days, and the EtOAc extracts from YPM medium exhibited the most significant antibacterial activity(Table 1).

3.2. Identification of MBL39

The isolate MBL39 shows 100% similarity with *Streptomyces californicus* NBRC 12750(GeneBank accession No. AB184116.2), *Streptomyces griseus* subsp. rhodochrous NBRC 13849 (GeneBank accession No. AB184821.1) and *Streptomyces* sp. 82035(GeneBank accession No. AY996837.1)(Fig. 2). However,

it is not certain that MBL39 is *Streptomyces californicus*, *Streptomyces griseus* subsp. rhodochrous or *Streptomyces* sp. 82035. There needs to be another way to identify this strain more exactly.

3.3. Antimicrobial activity of compounds from MBL39

The in vitro antimicrobial activities of compounds from MBL39 were assessed against three representative Gram-positive baceria; *Staphylococcus aureus* (ATCC 6538p), *Bacillus subtilis* (ATCC 6633), *Kocuria rhizophila* (NBRC 12708), three Gram-negative bacteria; *Salmonella enterica* (ATCC 14028), *Proteus hauseri* (NBRC 3851), *Escherichia coli* (ATCC 35270). Among the tested organic layer, an aliquot of EtOAc layer exhibited stronger inhibitory activity than that of other organic layers(Table 2). The EtOAc extract was subjected to C_{18} reversed-phase vacuum flash chromatography, and 100% MeOH fraction showed the most significant inhibitory activity than that of other fractions(Table 3). Thus, the 100% MeOH fraction was separated by reversed-phase column with 90% aqueous

methanol to yield 0.3, 0.5, 0.7, 1.0, 0.7, 0.9, 0.4 mg of 1-7, respectively(Fig. 4). After isolation with HPLC, compound 1, 2, 4 and 5 exhibited antibacterial activities against Gram-positive and Gram-negative bacteria except *Escherichia coli*(Table 4). In antifungal activity assay, all of the compounds 1-7 did not inhibit fungal cell growth at 100 μg/ml (data not shown).

3.4. ICL inhibition activity

The effect of isolated compounds 1-7 on ICL of *C. albicans* was evaluated. The inhibitory potencies(IC_{50}) of the tested compounds are shown in Table 5 and are compared to that of a known ICL inhibitor, 3-nitropopinate. All compounds did not exhibit inhibitory activity against isocitrate lyase(ICL). In case of compound 4, the initial value was so high that the result cannot be trusted.

3.5. NMR & MS analysis

The molecular formula $C_{18}H_{35}NO$ was deduced by low resolution FAB mass spectrum($[M + H]^+$ at *m/z* 282) for compound 4(Fig. 11). EI mass spectrum indicated the characteristic fragmentation of aliphatic amides(Fig. 12). Peak at *m/z* 59 corresponds to $[H_2C=C-NH_2-OH^+]$ and is characteristic of all fatty primary amides. Peak at *m/z* 72 corresponds to $[H_2C=CH-C-NH_2-OH^+]$ and is found for synthetic oleamide (27). ^1H and ^{13}C NMR data assigned for this compound by COSY, HSQC, HMBC and DEPT experiments were consistent with this formula(Fig. 5 ~ 10). The ^1H NMR showed two olefinic methines at δ_H 5.33, α-methylene protons of oleamide at δ_H 2.01, and methyl protons at δ_H 0.89, respectively. The ^{13}C NMR data indicated amide carbonyl carbon at 179.5 ppm and two olefinic carbons at 131.0 and 130.9 ppm, respectively. Based on the results of combined spectroscopic analysis and comparison of the spectral data with the previously reported data, the structure of compound 4 was identified to be oleamide(Table 6).

Compound 3 was analyzed for $C_{16}H_{33}NO$ by FABMS and EI mass spectrum of it corresponds to hexadecanamide(Fig. 13 ~ 15). Therefore, compound 3 was expected as hexadecanamide.

4. Discussion

Fatty acid amides are widespread in nature. They were incorporated into some lipid molecules such as ceramides, glyco-sphingolipids, *N*-acylated lipids, and bacterial lipoproteins. They are lipid bioregulators formed from long chain saturated and unsaturated fatty acids via amidation by the corresponding amines. Fatty acid amides are also found in grasses and microalgae. Kawasaki have isolated two natural fatty amides - hexadecanamide and octadecanamide from the shoots of marine grass *Zostera marina*(28). Octadecanamide and other fatty amides were isolated from cyanobacterium *Aphannizomenon flos-aquae*(29). Malyngamides, derivatives of tetradecanoic acid, were isolated from cyanobacterium *Lyngbya majuscula*(30). The cyclopropyl fatty amide, grenadamide, was detected in *Lyngbya majuscula*(31). Branched-chain fatty amide was isolated from epiphytic dinoflagellate *Coolia monotis*(32).

Oleamide is an amide of the fatty acid oleic acid, and it occurs naturally in the body of animals. It was originally characterized as an endogenous bioactive substance, isolated from the cerebrospinal fluid of sleep deprived cats(22). It was characterized in 1995 by Benjamin Cravatt III and Richard Lerner at the Scripps Research Institute in La Jolla, CA. It

accumulates in the cerebrospinal fluid during sleep deprivation and induces sleep in animals(23).

Intriguingly, later studies found that oleamide levels in the brain of the ground squirrel were 2.5-fold higher in hibernating animals relative to that found in non-hibernating animals. Other functions ascribed to oleamide, since its discovery as a sleep-inducing primary fatty acid amides, include the ability to modulate gap junction communication in glial cells, tracheal epithelial cells, seminiferous tubule cells, and fibroblasts, to allosterically activate the $GABA_A$ receptors and specific subtypes of the serotonin receptor, to effect memory processes, to increase food intake, to reduce anxiety and pain, to depress body temperature and locomotor activity, to stimulate Ca(II) release, and to relax blood vessels(42). It is being studied as a potential medical treatment for mood and sleep disorders, and cannabinoid-regulated depression(24). The mechanism of action of oleamide's sleep inducing effects is an area of current research.

It is likely that oleamide interacts with multiple neurotransmitter systems(20, 25). Oleamide is structurally related to the endogenous cannabinoid anandamide, and has the ability to bind to the CB1 receptor as a full agonist. Oleamide is broken down by fatty acid amide hydrolase (FAAH) enzymes, which also degrade the endocannabinoid anandamide. Exogenous oleamide's effects on locomotor activity and sleep are broadly

cannabinomimetic. However, oleamide binds only weakly to CB1 receptors and it cannot directly alter GTPγS binding. A popular interpretation of the above evidence is that oleamide competes with anandamide for FAAH, causing the levels of anandamide to increase thus producing sleep(43).

It has been reported that mice treated with oleamide before scopolamine injection were protected from scopolamine-induced memory or cognitive impairment in the passive avoidance test(21). It also has been reported that oleamide have antimicrobial activity(26). Thus, Oleamide should be used as a slip agent, a chemopreventive agent against Alzheimer's disease and an antibacterial agent.

In this study, the structural conciseness of six compounds are remained to be studied. They are expected as analogues of fatty acid amides. For the more detailed structures of these compounds, NMR analysis should be performed.

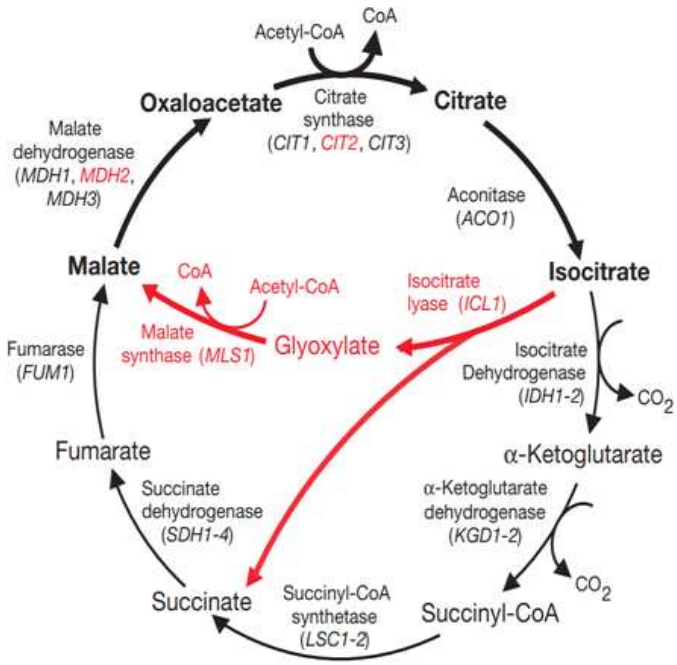

Fig. 1. Glyoxylate and tricarboxylic acid cycles

The glyoxylate cycle is an anaplerotic pathway of the tricarboxylic acid (TCA) cycle that allows growth on C_2 compounds by bypassing the CO_2-generating steps of the TCA cycle. The unique enzymes of this route are isocitrate lyase (ICL) and malate synthase (MLS). ICL cleaves isocitrate to glyoxylate and succinate, and MLS converts glyoxylate and acetyl-CoA to malate. The end products of the bypass can be used for gluconeogenesis and other biosynthetic processes.

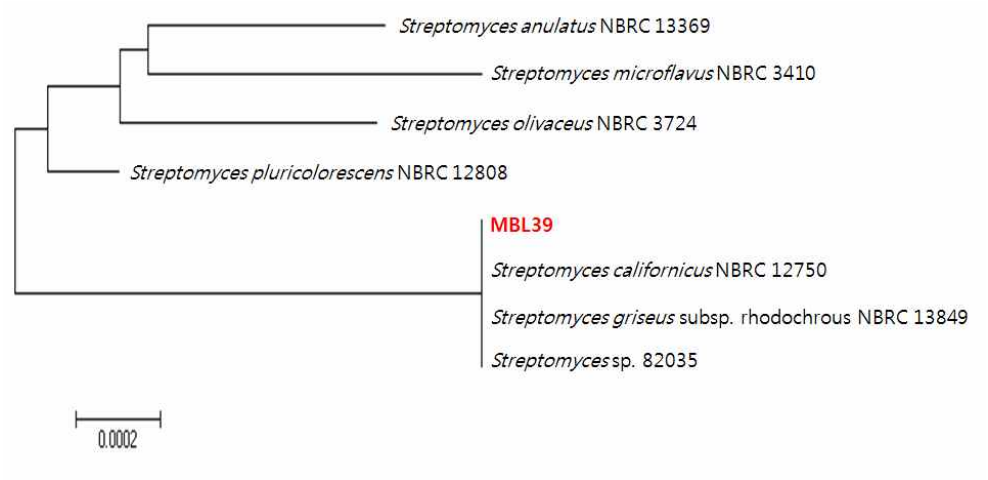

Fig. 2. Phylogenetic analysis of MBL39

The strain MBL39 shows 100% similarity with *Streptomyces californicus* NBRC 12750(GeneBank accession No. AB184116.2), *Streptomyces griseus* subsp. rhodochrous NBRC 13849 (GeneBank accession No. AB184821.1), and *Streptomyces* sp. 82035(GeneBank accession No. AY996837.1).

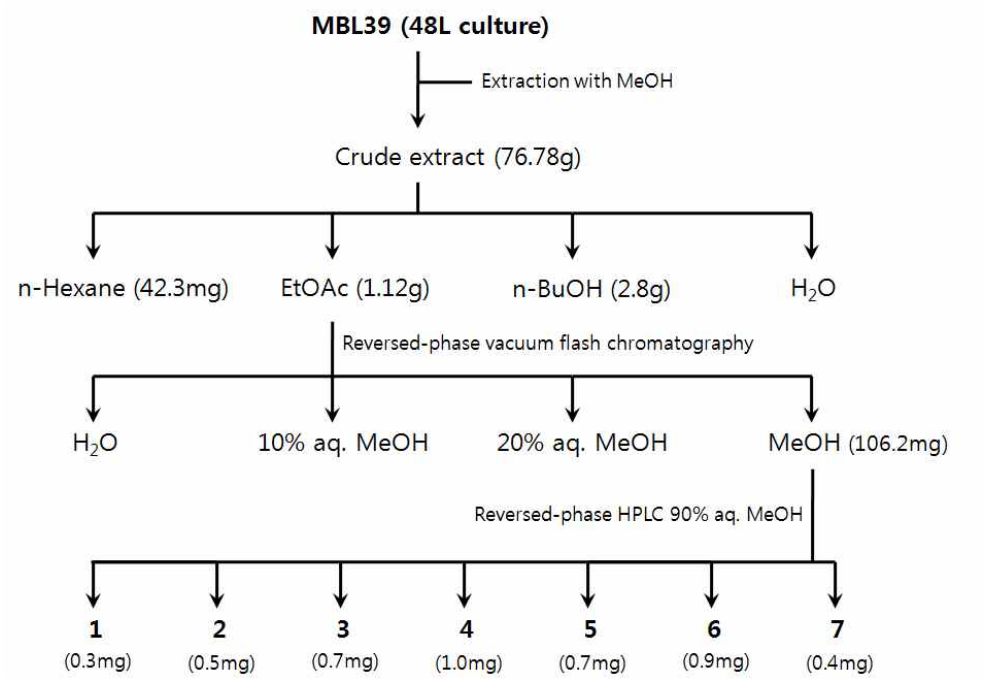

Fig. 3. Isolation procedure of compounds 1−7

Tabel 1. Antimicrobial activity of EtOAc extracts from each medium

EtOAc extract	MIC (μg/ml)			
	Bacteria		Fungi	
	A	B	C	D
GTYB	31.2	31.2	>100	>100
Bennett	15.6	31.2	>100	>100
GPY	15.6	15.6	>100	>100
SYP	15.6	31.2	>100	>100
M2	15.6	15.6	>100	>100
YPM	0.8	0.8	>100	>100
ampicillin	0.4	0.4	ND	ND
amphotericin B	ND	ND	0.4	1.6

A : *Staphylococcus aureus* ATCC 6538p, B : *Proteus hauseri* NBRC 3851,
C : *Candida albicans* ATCC 10231, D : *Aspergillus fumigates* HIC 6094
ND : not determined

Table 2. Antibacterial activity of dried aliquots of organic layer

organic layer	MIC (μg/ml)	
	Gram(+) bacteria	Gram(-) bacteria
	A	B
hexane	>100	>100
ethyl acetate	0.8	0.8
butanol	125	125
ampicillin	0.4	0.4

A : *Staphylococcus aureus* ATCC 6538p, B : *Proteus hauseri* NBRC 3851

Table 3. Antibacterial activity of each fraction from MBL39

fraction	MIC (μg/ml)	
	Gram(+) bacteria	Gram(-) bacteria
	A	B
H_2O	>100	>100
10% aq. MeOH	>100	>100
20% aq. MeOH	25	25
MeOH	0.8	0.8
ampicillin	0.4	0.4

A : *Staphylococcus aureus* ATCC 6538p, B : *Proteus hauseri* NBRC 3851

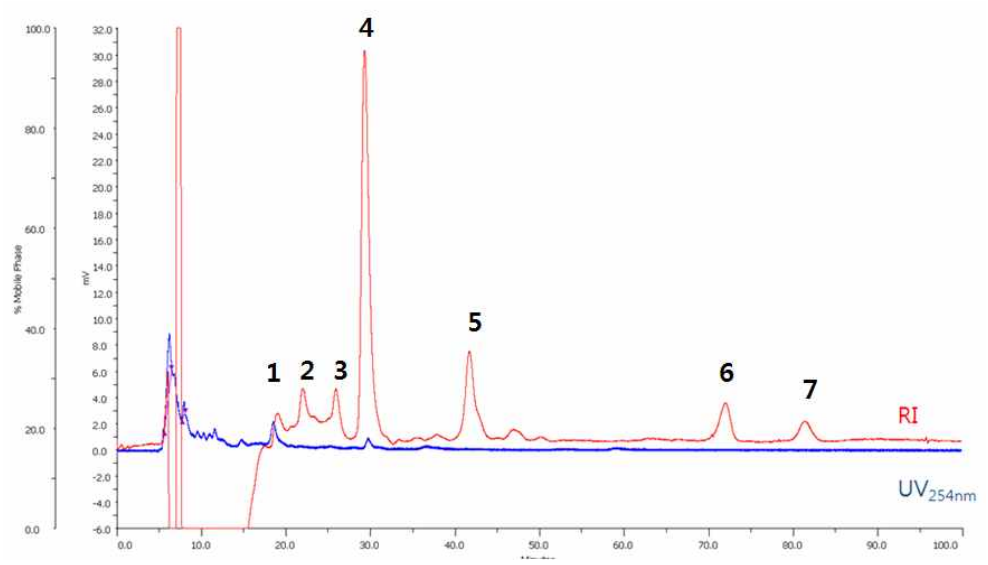

Fig. 4. HPLC chromatogram of compounds 1-7

Agilent Eclipse Plus column (250 mm × 4.6 mm) under isocratic conditions (90% aqueous methanol) at a flow rate of 0.5 ml/min, with refractive index detection

Table 4. Antibacterial activity of compounds 1−7

compound	MIC (μg/ml)					
	Gram(+) bacteria			Gram(-) bacteria		
	A	B	C	D	E	F
1	12.5	12.5	12.5	25	12.5	>50
2	12.5	25	12.5	25	12.5	>50
3	>50	>50	>50	>50	>50	>50
4	25	25	25	50	25	>50
5	6.3	6.3	3.1	12.5	6.3	>50
6	>50	>50	>50	>50	>50	>50
7	>50	>50	>50	>50	>50	>50
ampicillin	0.4	0.4	0.4	0.8	0.4	6.3

A : *Staphylococcus aureus* ATCC 6538p, B : *Bacillus subtilis* ATCC 6633,
C : *Kocuria rhizophila* NBRC 12708, D : *Salmonella enterica* ATCC 14028,
E : *Proteus hauseri* NBRC 3851, F : *Escherichia coli* ATCC 35270

Table 5. ICL inhibitory activity of compounds 1-7

compound	ICL IC_{50} (μg/ml)
1	>100
2	>100
3	>100
4	65.05
5	>100
6	>100
7	>100
3-nitropropinate	4.49

3-Nitropropionate was used as positive control of inhibitor of ICL.

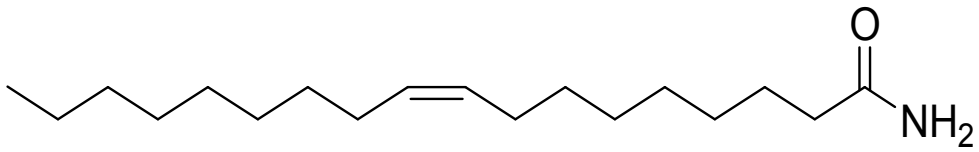

Table 6. ^{1}H and ^{13}C NMR assignment for compounds 4 in CD_3OD

Position	^{1}H	^{13}C
1	–	179.5
2	2.18 (t, 7.5)	36.7
3	1.60 (dq, 7.5, 7.5)	27.1
4	1.23–1.32 (m)[a]	30.7[a]
5	1.23–1.32 (m)[a]	30.6[a]
6	1.23–1.32 (m)[a]	30.5[a]
7	1.23–1.32 (m)[a]	31.0
8	2.01 (dt, 7.5, 7.5)	28.3
9	5.33 (t, 5.0)	130.9
10	5.33 (t, 5.0)	131.0
11	2.01 (dt, 7.5, 7.5)	28.3
12	1.23–1.32 (m)[a]	31.0
13	1.23–1.32 (m)[a]	30.5[a]
14	1.23–1.32 (m)[a]	30.5[a]
15	1.23–1.32 (m)[a]	30.4[a]
16	1.23–1.32 (m)[a]	33.2
17	1.23–1.32 (m)[a]	23.9
18	0.89 (t, 7.0)	14.6

[a]Assignments may be interchanged.

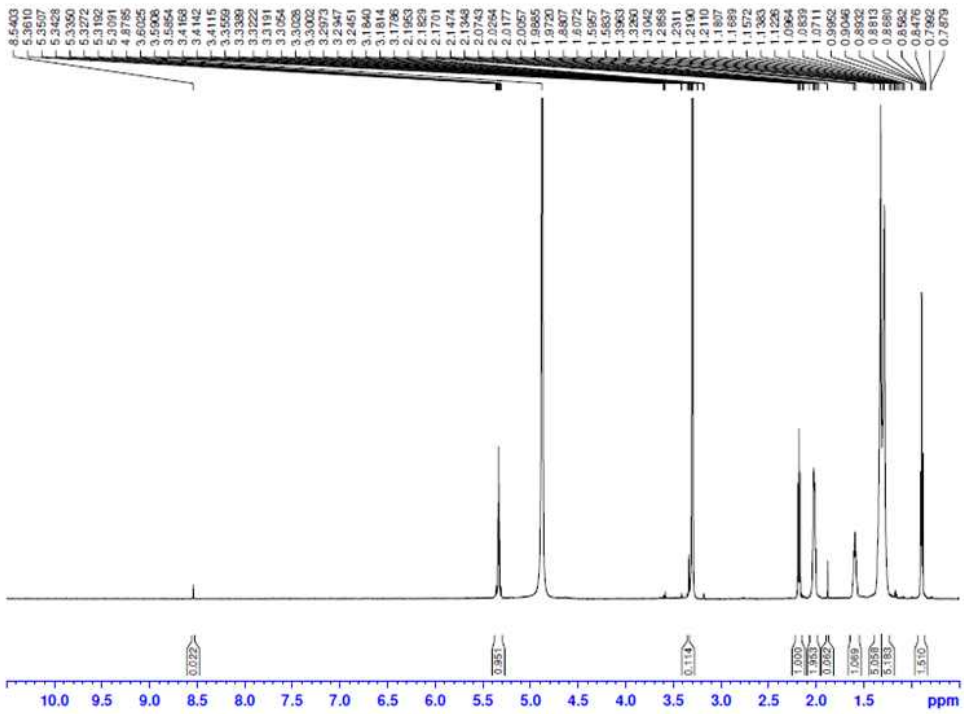

Fig. 5. ^{1}H spectrum of compound 4

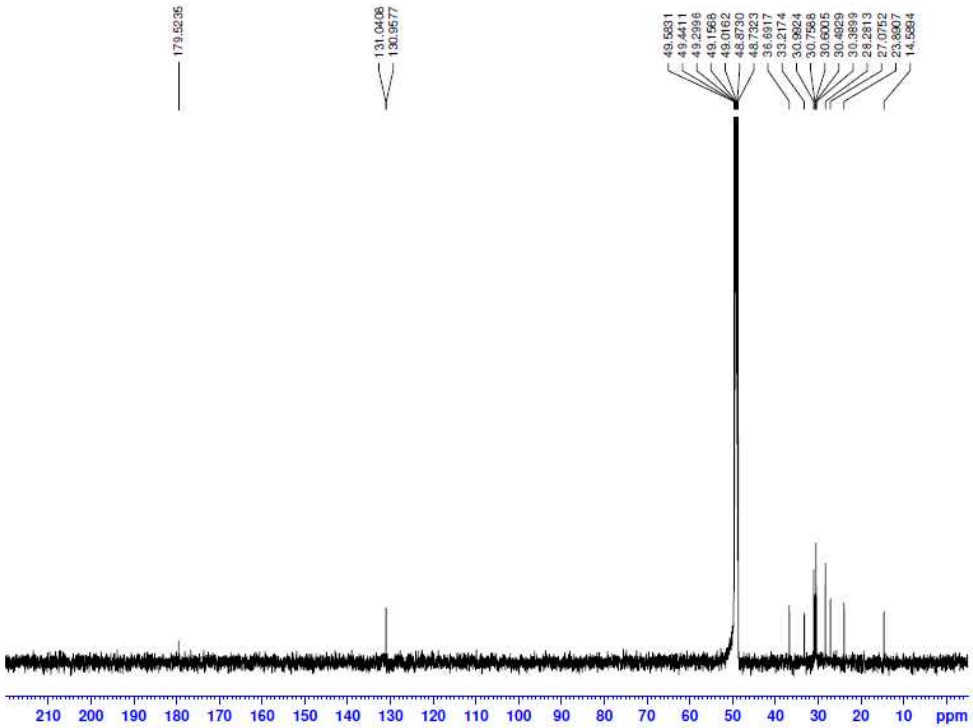

Fig. 6. ^{13}C spectrum of compound 4

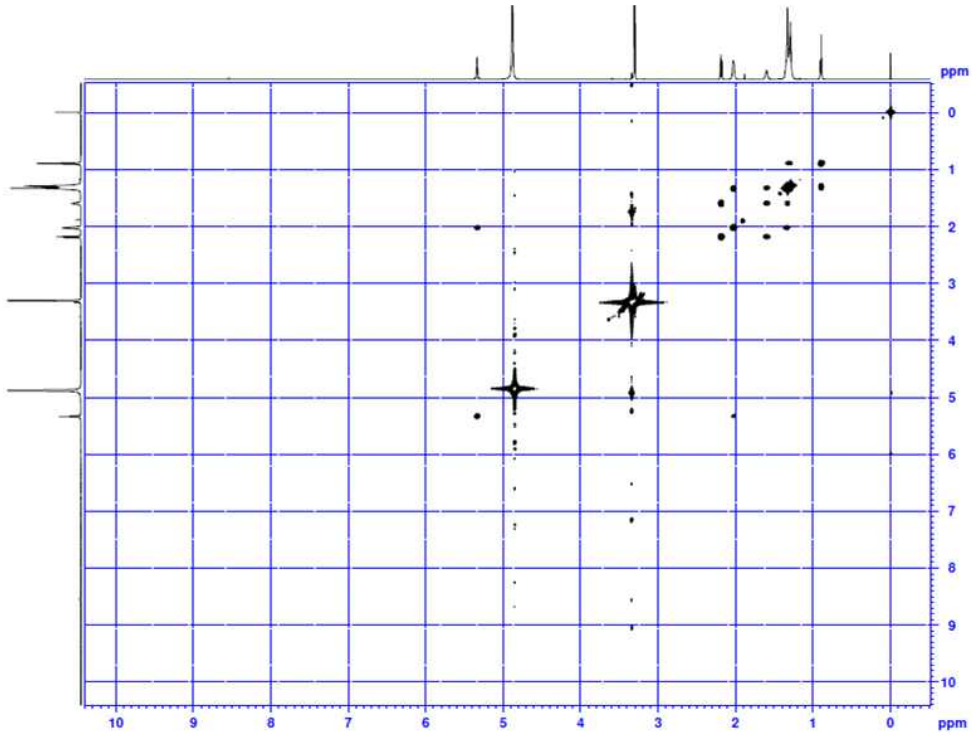

Fig. 7. $^{1}H-^{1}H$ COSY spectrum of compound 4

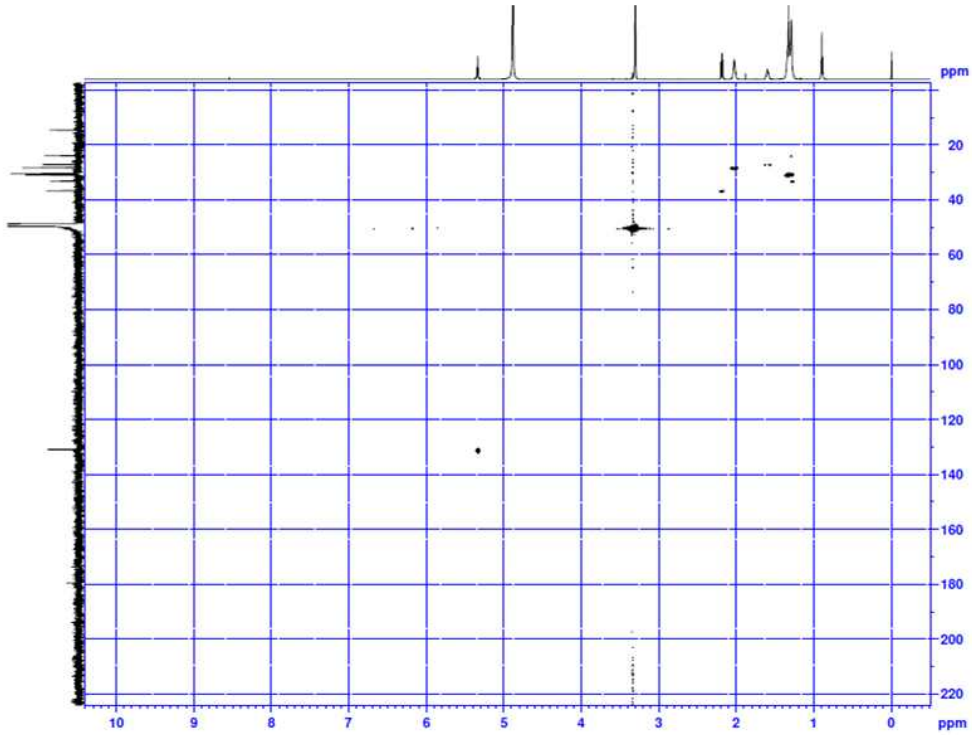

Fig. 8. HSQC spectrum of compound 4

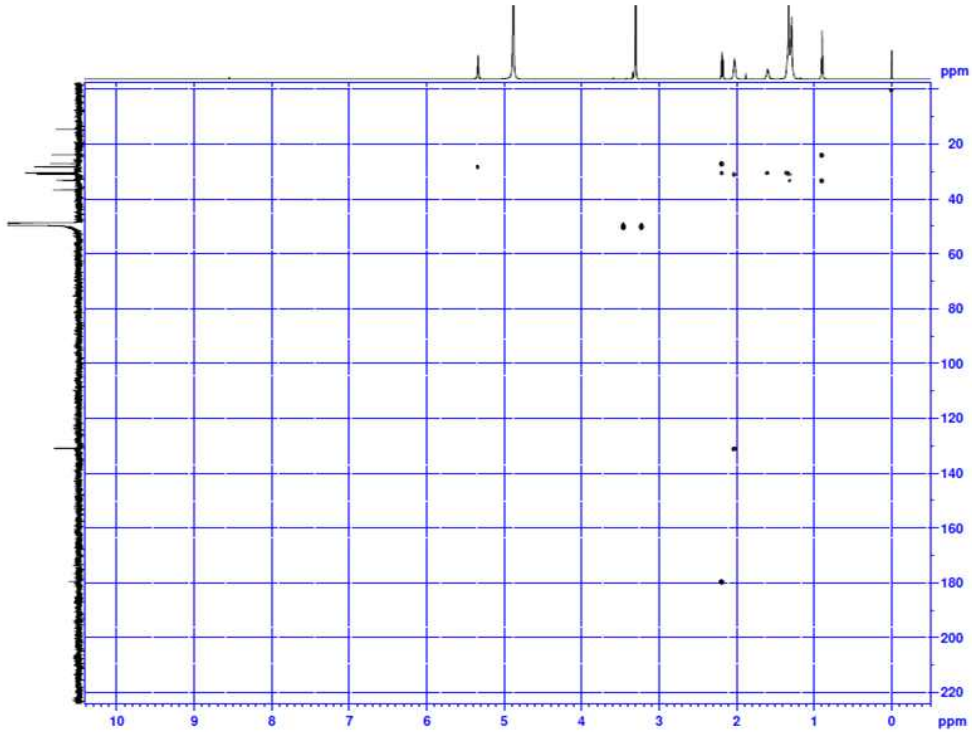

Fig. 9. HMBC spectrum of compound 4

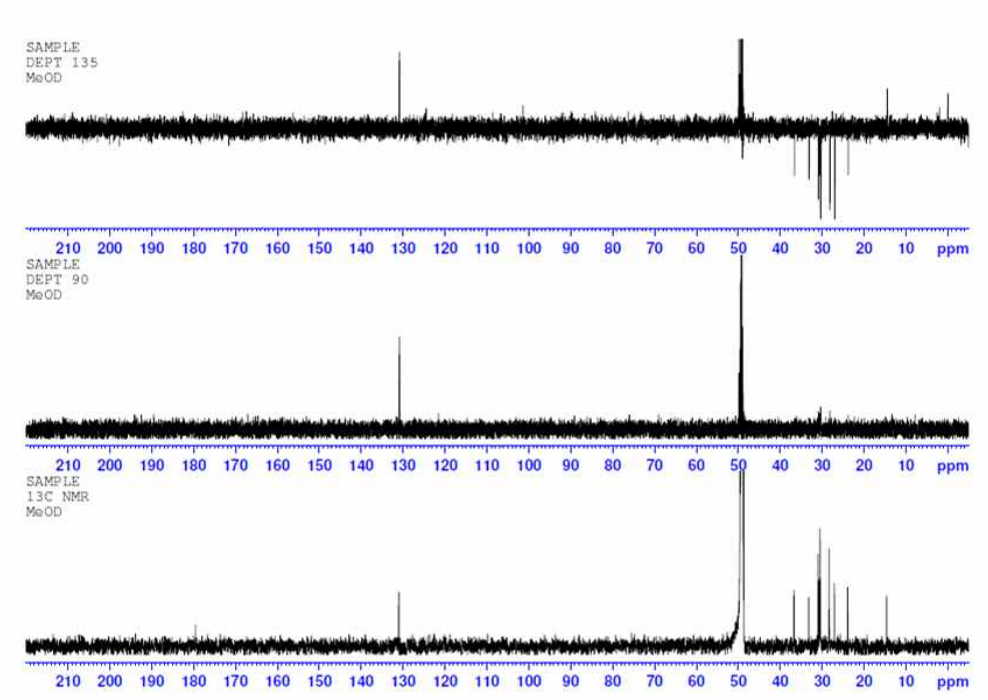

Fig. 10. DEPT spectrum of compound 4

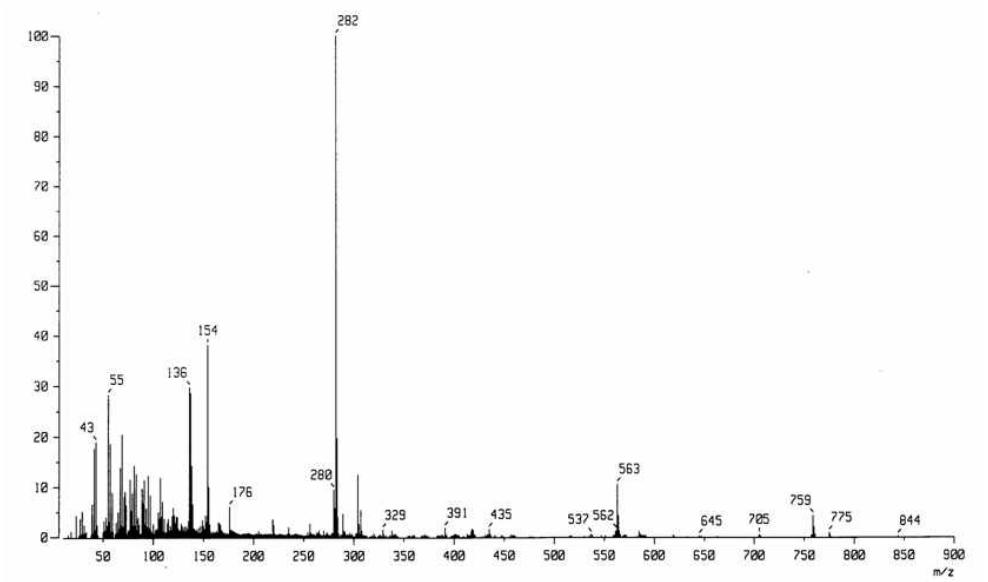

Fig. 11. FAB mass spectrum of compound 4

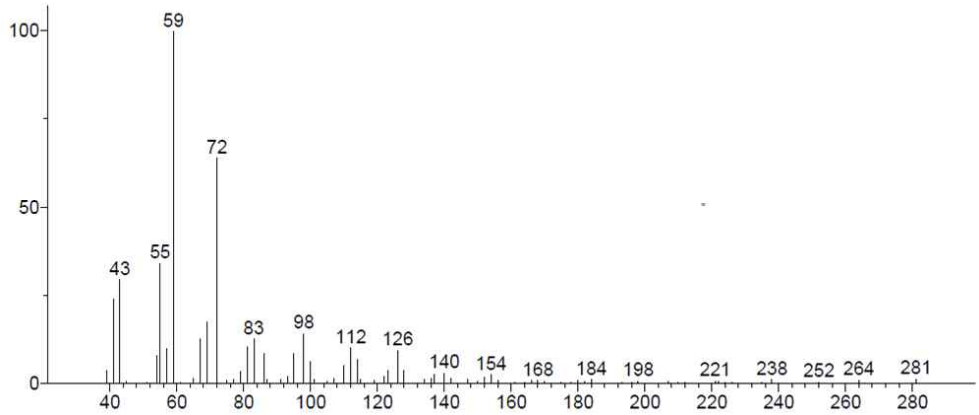

Fig. 12. EI mass spectrum of compound 4

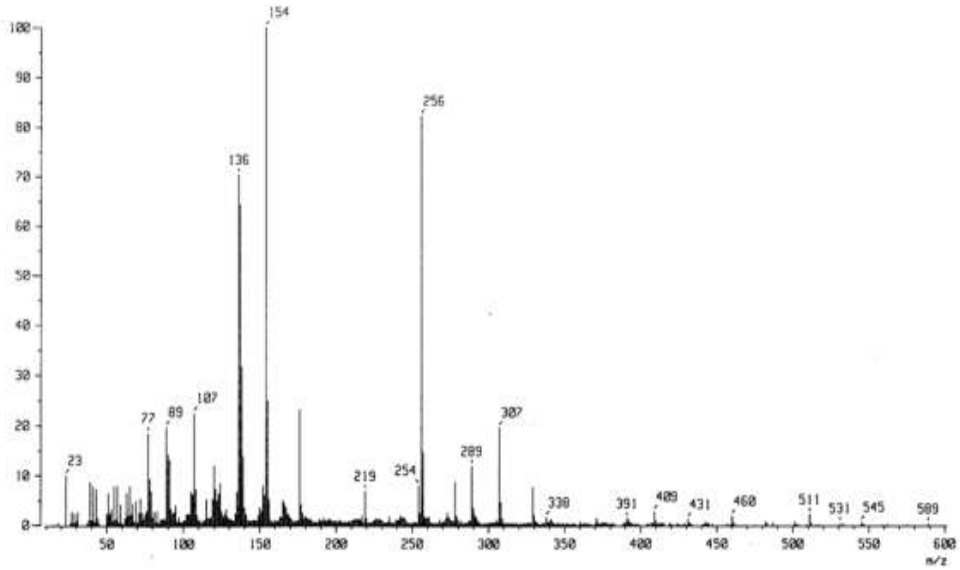

Fig. 13. FAB mass spectrum of compound 3

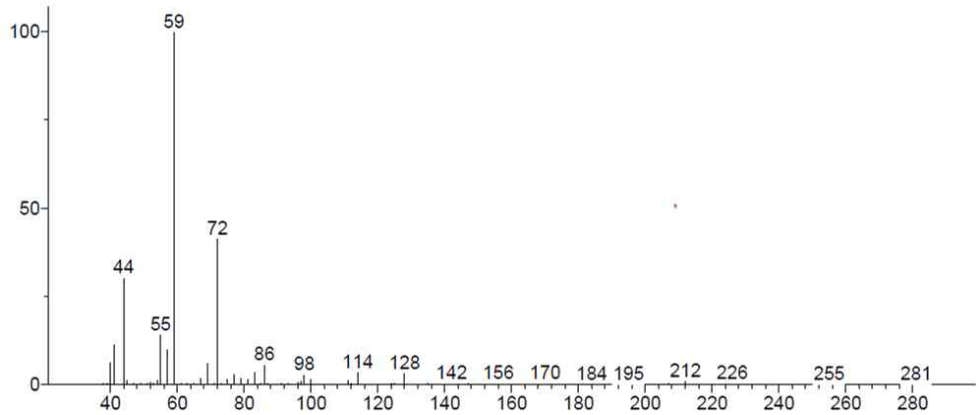

Fig. 14. EI mass spectrum of compound 3

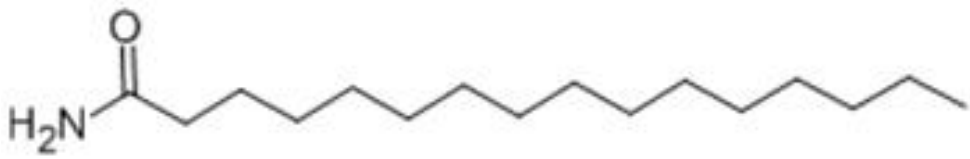

Fig. 15. Expected structure of compound 3

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Abstract in Korean

해양방선균 *Streptomyces* sp. MBL39 유래의 항균활성물질 연구

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해양생물 유래의 2차 대사산물은 다양한 생리활성을 갖고 있어 의학적 활용가치가 매우 높다. 새로운 생리활성 물질을 찾기 위해, 마이크로네시아에서 채취한 77개의 해양시료로부터 총 373종의 해양미생물을 분리하였다. 그 중 항미생물활성이 가장 뛰어난 한 균주를 16S rRNA 염기서열 분석을 통해 *Streptomyces* sp. MBL39로 동정하였다. *Streptomyces* sp. MBL39의 배양을 위해 6가지 배양배지 중 항세균활성이 가장 높게 나타난 YPM배지를 사용하였다. MBL39균주의 배양액을 감압농축하여 메탄올 조추출물을 얻은 후, 이것을 다시 물에 녹여 그

수용액을 hexane, ethyl acetate, butanol과 차례로 용매분획을 실시하였다. 이 중 EtOAc층에서 항세균활성이 확인 되어, 이 층으로부터 vacuum flash chromatography 및 HPLC를 통해 총 7개의 순수한 물질을 분리하였다. 이 중 물질 1, 2, 4, 5는 3.1-25 μg/ml의 MIC값을 갖는 항세균활성이 있는 것으로 나타났다. 또한, 미생물 효소인 ICL 및 SrtA에 대한 저해활성을 확인하였다. 물질 4의 구조는 여러 가지 분광스펙트럼 분석법을 이용하여 oleamide로 동정하였다. 추가적으로 물질 1, 2, 5의 구조 분석을 위해 NMR분석이 수행될 것이다.

주요어 : Marine actinomycete, *Streptomyces*, Oleamide,
Antibacterial activity

학번 : 2011-21287